MEMORANDUM

DATE: September 13, 2007

SUBJECT: Secondary Review of Contractor's (DynCorp Systems & Solutions LLC, a CSC Company) Efficacy Review for Axen 30;
EPA Reg. No. 72977-3;
DP Barcode: D341465

FROM: Lorilyn M. Montford
Product Science Branch
Antimicrobials Division (7510P)

THRU: Tajah Blackburn, Ph.D., Acting Team Leader
Product Science Branch
Antimicrobials Division (7510P)

Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510P)

TO: Marshall Swindell, PM 33/ Karen Leavy
Regulatory Management Branch I
Antimicrobials Division (7510P)

APPLICANT: ETI H2O, Inc.
1725 Gillespie Way
El Cajon, CA 92020

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Silver	0.003%
Citric Acid	4.840%
Other Ingredients	95.157%
Total	100.000%

I BACKGROUND

The product, Axen 30 (EPA Reg. No. 72977-3), is an EPA-approved, ready-to-use, disinfectant (bactericide, fungicide, virucide) and deodorizer for use on hard, non-porous surfaces in household, commercial, institutional, industrial, food preparation, animal care, and hospital or medical environments. The applicant requested to amend the product registration to add claims for effectiveness as a disinfectant against *Acinetobacter baumannii*, *Campylobacter jejuni*, *Clostridium difficile*, *Staphylococcus aureus* – CA-MRSA, *Staphylococcus aureus* – CA-MRSA PVL Positive, Avian Influenza A (H3N2) virus, Norwalk virus (as Feline calicivirus), Human coronavirus, and Rotavirus. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant's representative to the Agency (dated May 24, 2007), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-5 (Notice of Supplemental Distribution of a Registered Pesticide Product), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), ten studies (MRID 471380-01 through 471380-10), Statements of No Data Confidentiality Claims for all ten studies, and the proposed label.

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces, including: activity centers, appliances, bed frames, cabinets, changing tables, computer keyboards, counters, diaper pails, doorknobs, equipment tables, exterior toilet and urinal surfaces, faucet handles, floors, furniture, grocery carts, hand rails, jungle gyms, laundry hampers, light switch covers, sinks, strollers, showers, tanning beds, telephones, toys, tubs, walls, waste containers, and wheelchairs. Directions on the proposed label provided the following information regarding use of the product:

> As a disinfectant against bacteria: Pre-clean surfaces. Wet surfaces completely with the product. Surfaces must remain wet for 2 minutes. Wipe surfaces dry with a clean towel, if desired.
>
> As a disinfectant against viruses: Pre-clean surfaces. Wet surfaces completely with the product. Surfaces must remain wet for 10 minutes. Wipe surfaces dry with a clean towel, if desired.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria)

Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants

Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step. These Agency standards are presented in DIS/TSS-1.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Virucides – Novel Virus Protocol Standards

To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 product lots per laboratory).

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 471380-01 "AOAC Use-Dilution Method, Test Organism: Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA" for Axen 30, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – March 19, 2007. Project Number A04700.

This study was conducted against Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA (NRS 123) (Genotype USA400) (obtained from the NARSA Contracts Administrator at Focus Technologies, Inc., Herndon, VA). Two lots (Lot Nos. 2006.003.001 and 2007.008.002) of the product, Axen 30, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. The product was received ready-to-use. Testing was conducted on February 22, 2007 and March 7, 2007. [The product was not tested in the presence of an organic soil load.] Ten (10)

stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 35-37°C at 40% relative humidity. Each carrier was exposed to 10 mL of the product for 2 and 5 minutes at 20±1°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecitin and 0.5% Tween 80 to neutralize. For testing conducted on March 7, 2007, individual carriers were transferred from primary subculture tubes to secondary subculture tubes containing 10 mL of Letheen Broth with 0.07% Lecitin and 0.5% Tween 80 at least 30 minutes after the primary subculture. All subcultures were incubated for 48±4 hours at 35-37°C. Subcultures prepared on February 22, 2007 were stored for 2 days at 2-8°C prior to examination. Following incubation, or incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: Testing performed on February 22, 2007 demonstrated growth of the test organism in one product lot (Lot No. 2006.003.001) at a 2-minute contact time. Testing of the product lot was repeated on March 7, 2007 to evaluate for false positives.

Note: Antibiotic resistance of Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition confirmed antibiotic resistance of Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA to oxacillin. See page 9 and Table 5 of the laboratory report.

Note: ATS Laboratory Protocol IMS01020807.UD.1, included as part of the laboratory report, is marked as "Proprietary Information."

2. MRID 471380-02 "AOAC Use-Dilution Method, Test Organism: *Clostridium difficile* (ATCC 9689)" for Axen 30, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – March 12, 2007. Amended report date – March 19, 2007. Project Number A04690.

This study was conducted against *Clostridium difficile* (ATCC 9689). Two lots (Lot Nos. 2006.003.001 and 2007.008.002) of the product, Axen 30, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. The product was received ready-to-use. [The product was not tested in the presence of an organic soil load.] Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 20±4 hour old suspension of the test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried in an anaerobic chamber for 40 minutes at 35-37°C at 23.8% relative humidity. Each carrier was exposed to 10 mL of the product for 30 seconds and 2 minutes at 20±1°C. Following exposure, individual carriers were transferred to 10 mL of Fluid Thioglycollate Medium with 0.07% Lecithin and 0.5% Tween 80 to neutralize. Individual carriers were then transferred to secondary subculture tubes containing 10 mL of Fluid

Thioglycollate Medium at least 30 minutes after the primary subculture. All subcultures were incubated in an anaerobic chamber for 2 days at 35-37°C and then stored at 2-8°C for 2 days prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and verification of spores present in the initial suspension.

Note: The original report was amended to correct various typographical errors.

Note: ATS Laboratory Protocol IMS01020807.UD.2, included as part of the laboratory report, is marked as "Proprietary Information."

3. MRID 471380-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus" for Axen 30, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – August 18, 2006. Project Number A04151.

This study, under the direction of Study Director Kelleen Gutzmann, was conducted against Feline calicivirus (F-9 strain; ATCC VR-782) using CRFK cells (Crandel Reese feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. Two lots (Lot Nos. 2006.003.001 and 2005.269.001) of the product, Axen 30, were tested according to ATS Lab Protocol No. IMS01010906.FCAL.2 (copy not provided). The product was received ready-to-use. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 mL of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 49% relative humidity. For each product lot, two dried virus films were sprayed (3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The subcultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of specified cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus titer, dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The $\log_{10}$ reduction in infectivity was also calculated using the revised EPA approved method for calculating the Most Probable Number (MPN).

4. MRID 471380-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus – Confirmatory Assay" for Axen 30, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 17, 2006. Amended report date – August 24, 2006. Project Number A04150.

This study, under the direction of Study Director Mary J. Miller, was conducted against Feline calicivirus (F-9 strain; ATCC VR-782) using CRFK cells (Crandel Reese feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. One lot (Lot No. 2005.269.001) of the product, Axen 30, was tested according to ATS Lab Protocol No. IMS01010906.FCAL.1 (copy not provided). The product was received ready-to-use. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 mL of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.1°C at 49% relative humidity. For the single product lot, two dried virus films were sprayed (3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 10 minutes at 20.1°C. The plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The subcultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of specified cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus titer, dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The $\log_{10}$ reduction in infectivity was also calculated using the revised EPA approved method for calculating the Most Probable Number (MPN).

Note: The original report was amended to correct a typographical error.

5. MRID 471380-05 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human Coronavirus" for Axen 30, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – March 9, 2007. Project Number A04704.

This study was conducted against Human coronavirus (Strain 229E; ATCC VR-740) using WI-38 cells (human lung cells; ATCC CCL-75; propagated in-house) as the host system. Two lots (Lot Nos. 2006.003.001 and 2007.008.002) of the product, Axen 30, were tested according to ATS Labs Protocol No. IMS01020807.HCV (dated February 8, 2007). The product was received ready-to-use. The stock virus culture was adjusted to contain 1% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 mL of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 54% relative humidity. For each product lot, one dried virus film was sprayed (3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 3 and 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. WI-38 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The subcultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 9 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls

included those for dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: ATS Laboratory Protocol IMS01020807.HCV, included as part of the laboratory report, is marked as "Proprietary Information."

6. MRID 471380-06 "AOAC Use-Dilution Method, Test Organism: Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA, PVL Positive" for Axen 30, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – May 8, 2007. Project Number A04890.

This study was conducted against Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA, PVL Positive (NARSA #NRS192) (obtained from the NARSA Contracts Administrator at Focus Technologies, Inc., Herndon, VA). Two lots (Lot Nos. 2006.003.001 and 2007.008.002) of the product, Axen 30, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. The product was received ready-to-use. [The product was not tested in the presence of an organic soil load.] Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 35-37°C at 40% relative humidity. Each carrier was exposed to 10 mL of the product for 2 and 5 minutes at 20±1°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80 to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA, PVL Positive was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition confirmed antibiotic resistance of Community Associated Methicillin Resistant *Staphylococcus aureus* – CA-MRSA, PVL Positive to oxacillin. See page 9 and Table 5 of the laboratory report.

Note: ATS Laboratory Protocol IMS01032207.UD, included as part of the laboratory report, is marked as "Proprietary Information."

7. MRID 471380-07 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rotavirus" for Axen 30, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – March 9, 2007. Project Number A04705.

This study was conducted against Rotavirus (Strain WA; obtained from the University of Ottawa, Ontario, Canada) using MA-104 cells (Rhesus monkey kidney cells; originally obtained from Diagnostic Hybrids Inc., Athens, OH; propagated in-house) as the host system. Two lots (Lot Nos. 2006.003.001 and 2007.008.002) of the product, Axen 30, were tested according to

ATS Labs Protocol No. IMS01020807.ROT (dated February 8, 2007). The product was received ready-to-use. The stock virus culture was adjusted to contain 1% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 mL of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 52% relative humidity. For each product lot, one dried virus film was sprayed (3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 3 and 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, 0.5 µg/mL Trypsin, and 2.0 mM L-glutamine. MA-104 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The subcultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

Note: ATS Laboratory Protocol IMS01020807.ROT, included as part of the laboratory report, is marked as "Proprietary Information."

8. MRID 471380-08 "AOAC Use-Dilution Method, Test Organism: *Acinetobacter baumannii* (ATCC 19606)" for Axen 30, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – March 12, 2007. Amended report date – May 8, 2007. Project Number A04701.

This study was conducted against *Acinetobacter baumannii* (ATCC 19606). Two lots (Lot Nos. 2006.003.001 and 2007.008.002) of the product, Axen 30, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. The product was received ready-to-use. [The product was not tested in the presence of an organic soil load.] Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 48-54 hour old suspension of the test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 40 minutes at 35-37°C at 40% relative humidity. Each carrier was exposed to 10 mL of the product for 2 and 5 minutes at 20±1°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecitin and 0.5% Tween 80 to neutralize. All subcultures were incubated for 48±4 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: The original report was amended to add the final report completion date and to remove the organic sterility control description.

Note: ATS Laboratory Protocol IMS01020807.UD.3, included as part of the laboratory report, is marked as "Proprietary Information."

9. MRID 471380-09 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Avian Influenza A (H3N2) virus (Avian Reassortant)" for Axen 30, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 24, 2006. Project Number A04153.

This study was conducted against Avian Influenza A (H3N2) virus (Avian Reassortant) (Strain A/Washington/897/80 X A/Mallard/New York/6750/78; ATCC VR-2072) using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc; maintained in-house) as the host system. Two lots (Lot Nos. 2006.003.001 and 2006.158.001) of the product, Axen 30, were tested according to ATS Labs Protocol No. IMS01050206.AFLU (copy not provided). The product was received ready-to-use. The stock virus culture contained 1% fetal bovine serum as the organic soil load. Films of virus were made by spreading 0.2 mL of virus inoculum on the bottoms of separate sterile, glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each product lot, one dried virus film was sprayed (3 pumps) with the product at a distance of 6-8 inches from the carrier surface. The carriers were allowed to remain wet for 10 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed through Sephadex columns, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of each dilution. The subcultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus titer, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

10. MRID 471380-10 "AOAC Use-Dilution Method, Test Organism: *Campylobacter jejuni* (ATCC 29428)" for Axen 30, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – March 20, 2007. Project Number A04702.

This study was conducted against *Campylobacter jejuni* (ATCC 29428). Two lots (Lot Nos. 2006.003.001 and 2007.008.002) of the product, Axen 30, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. The product was received ready-to-use. [The product was not tested in the presence of an organic soil load.] Ten (10) stainless steel penicylinder carriers per product lot were immersed in a 2-4 day old suspension of the test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 20 minutes at 25-30°C at 68% relative humidity. Each carrier was exposed to 10 mL of the product for 2 and 5 minutes at 20±1°C. Following exposure, individual carriers were transferred to 10 mL of Fluid Thioglycollate Medium with 0.07% Lecitin and 0.5% Tween 80 to neutralize. Individual carriers were then transferred to secondary subculture tubes containing 10 mL of Fluid Thioglycollate Medium at least 30 minutes after the primary subculture. All subcultures were incubated for 3 days at 35-37°C in 5.0% CO_2. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

Note: The applicant provided the data for a failed trial set up on February 23, 2007. In that trial, the neutralization control did not result in positive growth of the challenge microorganism. Testing was repeated on March 9, 2007. See page 8 and Attachment I of the laboratory report.

Note: ATS Laboratory Protocol IMS01020807.UD.4, included as part of the laboratory report, is marked as "Proprietary Information."

V RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Carrier Population (CFU/carrier)
		Lot No. 2006.003.001	Lot No. 2007.008.002	
30-Second Contact Time				
471380-02	*Clostridium difficile*	1° 0/10; 2° 0/10	1° 0/10; 2° 0/10	5.2×10^5
2-Minute Contact Time				
471380-01	*Staphylococcus aureus* – CA-MRSA Test Date: 02/22/07 Test Date: 03/07/07	 1/10 1° 0/10; 2° 0/10	 0/10 ---	 4.5×10^6 3.9×10^6
471380-02	*Clostridium difficile*	1° 0/10; 2° 0/10	1° 0/10; 2° 0/10	5.2×10^5
471380-06	*Staphylococcus aureus* – CA-MRSA, PVL Positive	0/10	0/10	9.1×10^6
471380-08	*Acinetobacter baumannii*	0/10	0/10	3.7×10^6
471380-10	*Campylobacter jejuni*	1° 0/10; 2° 0/10	1° 0/10; 2° 0/10	6.5×10^6
5-Minute Contact Time				
471380-01	*Staphylococcus aureus* – CA-MRSA Test Date: 02/22/07	 0/10	 0/10	 4.5×10^6
471380-06	*Staphylococcus aureus* – CA-MRSA, PVL Positive	0/10	0/10	9.1×10^6
471380-08	*Acinetobacter baumannii*	0/10	0/10	3.7×10^6
471380-10	*Campylobacter jejuni*	1° 0/10; 2° 0/10	1° 0/10; 2° 0/10	6.5×10^6

MRID Number	Organism	Results			Dried Virus Control ($TCID_{50}$/0.1 mL)
3-Minute Contact Time					
			Lot No. 2006.003.001	**Lot No. 2007.008.002**	
471380-05	Human coronavirus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.5}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
471380-07	Rotavirus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{5.75}$
		10^{-2} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥ 4.25 $\log_{10}$	≥ 4.25 $\log_{10}$	
10-Minute Contact Time					
471380-05	Human coronavirus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{4.75}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
471380-07	Rotavirus	10^{-1} dilution	Cytotoxicity	Cytotoxicity	$10^{6.0}$
		10^{-2} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.1 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	≥ 4.5 $\log_{10}$	≥ 4.5 $\log_{10}$	
			Lot No. 2006.003.001	**Lot No. 2005.269.001**	
471380-03	Feline calicivirus	10^{-1} to 10^{-4} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$ and $10^{4.75}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
471380-04	Feline calicivirus	10^{-1} dilution	---	Cytotoxicity	$10^{5.5}$ and $10^{5.25}$
		10^{-2} to 10^{-4} dilutions	---	Complete inactivation	
		$TCID_{50}$/0.1 mL	---	$\leq 10^{1.5}$	
		Log reduction	---	4.0 and 3.75 $\log_{10}$	
			Lot No. 2006.003.001	**Lot No. 2006.158.001**	
471380-09	Avian Influenza A (H3N2) virus	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

VI CONCLUSIONS

1. The submitted efficacy data support the use of the product, Axen 30, as a disinfectant on hard, non-porous surfaces against the following microorganisms under the following conditions:

Acinetobacter baumannii	2 and 5 minutes	MRID 471380-08
Campylobacter jejuni	2 and 5 minutes	MRID 471380-10
Clostridium difficile(vegetative)	30 seconds and 2 minutes	MRID 471380-02
Staphylococcus aureus – CA-MRSA	2 and 5 minutes	MRID 471380-01
Staphylococcus aureus – CA-MRSA, PVL Positive	2 and 5 minutes	MRID 471380-06

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. [Note that repeat testing was conducted against *Staphylococcus aureus* – CA-MRSA at a 2-minute contact time to evaluate for false positives.] Carrier population counts were at least 10^4. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Sterility controls did not show growth. Purity controls were reported as pure.

2. The submitted efficacy data support the use of the product, Axen 30, as a disinfectant with virucidal activity on hard, non-porous surfaces against the following microorganisms in the presence of a 1% organic soil load for the contact times listed:

Avian Influenza A (H3N2) virus	10 minutes	MRID 471380-09
Human coronavirus	3 and 10 minutes	MRID 471380-05
Rotavirus	3 and 10 minutes	MRID 471380-07

Recoverable virus titers of at least 10^4 were achieved. In studies against Rotavirus, cytotoxicity was observed in the 10^{-1} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against Avian Influenza A (H3N2) virus and Human coronavirus, complete inactivation (no growth) was indicated in all dilutions tested.

3. The submitted efficacy data (MRID 471380-03 and -04) support the use of the product, Axen 30, as a disinfectant with virucidal activity on hard, non-porous surfaces against Feline calicivirus (surrogate for Norovirus) in the presence of a 5% organic soil load for a contact time of 10 minutes. Recoverable virus titers of at least 10^4 were achieved. In the confirmatory study, cytotoxicity was observed in the 10^{-1} dilution. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In the initial study, complete inactivation (no growth) was indicated in all dilutions tested. Initial and confirmatory studies for Feline calicivirus were performed at the same laboratory but under different study directors. Confirmatory studies used one lot of product, not the standard two.

VII RECOMMENDATIONS

As in previous transmittals with the registrant, the Agency has stated that "kill time" and 'contact time" are synonymous terms. Therefore the contact time and the kill time must be consistent, as demonstrated by the efficacy studies submitted.

1. The proposed label claims that the product, Axen 30, is an effective disinfectant for use on hard, non-porous surfaces against the following microorganisms for the listed contact times:

Acinetobacter baumannii	2 minutes
Campylobacter jejuni	2 minutes
Staphylococcus aureus – CA-MRSA	2 minutes
Staphylococcus aureus – CA-MRSA, PVL Positive	2 minutes
Avian Influenza A	10 minutes
Human coronavirus	3 minutes
Norwalk virus (as Feline calicivirus)	10 minutes
Rotavirus	3 minutes

Data provided by the applicant support these claims. Please remove all references indicating that Human coronavirus is a "SARS surrogate." Acceptance of the data to support a label claim for Human coronavirus does not in any way support a label claim for the product as an effective disinfectant against the causative agent of Severe Acute Respiratory Syndrome (SARS). Furthermore claims for CA-MRSA are not acceptable, rather claims for MRSA, with appropriate designation are accepted by the Agency.

2. Claim for effectiveness against Clostridium difficile (vegetative cells) at a contact time of 30 seconds is unacceptable. The Agency has re-evaluated its acceptance of *Clostridium difficile* (vegetative cells) on previously accepted claims and requests for new claims. Peer-reviewed scientific literature and case studies have consistently demonstrated that the *C. difficile* spore is the source of *Clostridium difficile*-associated disease (CDAD), and is an immediate public health concern. In light of scientific guidance and supporting documentation, the Agency is certain that claims against the vegetative form of *C. difficile* are true statements, but are used in such a way as to give a false or misleading impression to the purchaser (40 CFR 156.10(a)(5)(vii), and therefore imply heightened efficacy. The Agency considers antimicrobial pesticides to be unique because of the critical nature of the threat to public health that may result from ineffective use of products due to obsolete or misleading labeling. As a result, any reference to claims of effectiveness against *Clostridium difficile* (vegetative cells) OR *Clostridium difficile* (without having supporting data against *C. difficile* spores) are unacceptable. To address the growing need for products in hospital/medical setting, the Agency is moving expeditiously to develop an appropriate test system and performance standards for *Clostridium difficile* (spores).

3. The proposed label states that the product, Axen 30, is effective against Respiratory syncytial virus [see page 5 of the proposed label]. Data was not provided to support this claim. The Data Matrix does not identify an efficacy study for this virus. Remove the reference to Respiratory syncytial virus, or provide efficacy data that fully meet DIS/TSS-7 requirements.

4. The following changes are required on the proposed label:

- Change "*Salmonella choleraesuis*" to read "*Salmonella enterica*" throughout the proposed label.

- Revise the organism listing on page 3 of the proposed label to include information about testing in the presence of an organic soil load as follows:

  Human coronavirus [2]
  Rotavirus [2]
  Avian Influenza A [2]
  Norwalk virus (as Feline calicivirus) [1]
  [1 Evaluated in the presence of 5% organic soil.]
  [2 Evaluated in the presence of 1% organic soil.]

- On page 2 of the proposed label, change "child car seats" to read "child car seats, hard surfaces only."

- On page 2 of the proposed label, change "lights switch covers" to read "light switch covers."

5. The list of ATCC numbers or other company designation numbers must be provided in one of the following locations:

- On the Data Matrix;
- On the master label (as optional text) with the listing of the organisms claimed; or
- As the final page of the master label (as optional text).

6. On the proposed label, change the "0" to "O" in Escherichia coli.

7. As consistent with appropriate nomenclature, the correct designation is "Norovirus" for "Norwalk virus" (Norovirus was recently approved as the official genus name for the group of viruses provisionally described as "Norwalk-like viruses" (NLV) (http://www.cdc.gov/ncidod/dhqp/id_norovirusFS.html).

8. The use of this product on children's toys requires a potable water rinse. Amend the proposed label to include this information.